

37. (new) A preparation according to claim 35, further comprising a fraction e) consisting of huperzine A.

B3
38. (new) A preparation according to claim 35, wherein fraction b) comprises phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine.

REMARKS

This application has been amended in a manner that is believed to place it in condition for allowance at the time of the next Official Action.

Claim 19 has been amended to recite that the long chain polyunsaturated fatty acids contain at least one member selected from the group consisting of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), dihomogammalinolenic acid (DHGLA), arachidonic acid (AA), linoleic acid and α -linoleic acid. Claim 19 further recites that the ratio of the total amount of EPA + DHA + DHGLA + AA to the total amount of linoleic acid and α -linoleic acid is above 0.4. Support for this recitation may be found at page 7, lines 5-7. Claims 20, 22 and 23 have been amended to more particularly point out and distinctly claim the present invention. New claims 34-38 have been added to recite that the composition of the present invention contains a fraction of long chain polyunsaturated fatty acids which contains Ω -3 and Ω -6 fatty acids wherein the ratio of Ω -3 fatty acids to Ω -6 fatty

acids is about 2.5 to 5.5 wt/wt. Support for new claims 34-38 may be found in the present specification at page 6, lines 20-26. It is believed that no new matter has been added to the present application.

In the outstanding Official Action, claims 22 and 23 were rejected under 35 USC 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. It is believed that the present amendment obviates this rejection.

The outstanding Official Action stated that the present specification does not provide support for the recitation of "gamma-3" and "gamma-6" fatty acids. Claims 22 and 23 have been amended to recite Ω -3 and Ω -6 fatty acids. Thus, it is believed that the claimed invention complies with the requirements of 35 USC 112, first paragraph.

Claim 20 was rejected under 35 USC 112, second paragraph, as allegedly being indefinite for failing to particularly point and distinctly claim the subject matter which applicant regards as the invention. This rejection is respectfully traversed.

The outstanding Official Action alleged that the phrase "citrate or citric acid" was indefinite. The Official Action

stated that it was unclear why both names for the same compound were provided. Claim 20 has been amended so as to delete the term "citrate" and insert the term --citrates--. Thus, it is believed that one of ordinary skill in the art would appreciate that the fraction may contain various citrates or citric acid. It is believed that claim 20 is definite to one of ordinary skill in the art.

In the outstanding Official Action, claims 19, 22, 25 and 33 were rejected under 35 USC 103(a) as allegedly being unpatentable over SAGAMI, HORROBIN and HASHIM. It is believed that the present amendment obviates this rejection.

It is respectfully submitted that the above-identified publications, alone or in combination with each other, fail to disclose or suggest the claimed invention. Applicants believe that the cited publications fail to recite each and every recitation of the claimed invention.

As the Examiner is aware, the SAGAMI publication is directed to the prevention and treatment of vascular diseases. A copy of the SAGAMI publication is enclosed with the present amendment. The SAGAMI publication teaches that lipids used in blood improving compositions that comprise phospholipids containing DHA can be administered to prevent or treat vascular diseases. The SAGAMI publication teaches that phospholipids extracted from cuttlefish may be used as the phospholipid component. The product thereby obtained can be added to a

variety of food products. The SAGAMI publication teaches that the food products may be soya processed foods that are rich in linoleic acids. Moreover, the SAGAMI publication teaches that ice creams that are rich in neutral triglycerides may be used (see page 5, paragraph 15).

However, the SAGAMI publication fails to disclose or suggest a composition comprising a fraction consisting of long chain polyunsaturated fatty acids that contain at least one member selected from the group consisting of EPA, DHA, DHGLA, AA, linoleic acid and α -linoleic acid wherein the ratio of the total amount of EPA + DHA + DHGLA + AA to the total amount of linoleic acid and α -linoleic acid is above 0.4. Moreover, the SAGAMI publication is completely silent as to a preparation having a long chain polyunsaturated fatty acid fraction containing Ω -3 and Ω -6 fatty acids wherein the ratio of Ω -3 fatty acids to Ω -6 fatty acids is about 2.5 to about 5.5 wt/wt.

The SAGAMI publication is for the use of food product which is rich in linoleic acid. Moreover, upon reviewing the SAGAMI publication, it is apparent that the amount of EPA + DHA + DHGLA + AA to the total amount of linoleic acid and α -linoleic acid reaches a maximum of 0.32.

Moreover, the SAGAMI publication advocates for the use of DHA-phospholipid and linoleic-rich types of products. Moreover, the SAGAMI publication teaches that the DHA-phospholipids may be incorporated in ice cream or other foods

rich in neutraltriglycerides. Applicants note that the present specification states that neutral triglycerides are preferably not included in the phospholipid fraction or in relatively low amounts, e.g., less than 40% and in particular less than 5% of the lipid fraction.

In an effort to remedy the deficiencies of the SAGAMI publication, the Examiner cites HORROBIN. HORROBIN relates to fruit juice enriched with polyunsaturated fatty acids, namely GLA and/or DGLA optionally with EPA, DHA, AA or SA for a diversity of treatments ranging from vascular diseases to skin disorders. The fatty acids are provided from a variety of compounds as disclosed on page 2, lines 32-33. However, it is believed that HORROBIN also fails to even mention or suggest the claimed long chain polyunsaturated fatty acids of the claimed invention.

Upon reviewing the HORROBIN disclosure, it is believed to be apparent that the amount of EPA + DHA + DHGLA + AA relative to the total amount of linoleic acid and α -linoleic acid is in a ratio well below 0.4. The HORROBIN publication does not even recognize that one of ordinary skill in the art should be cognizant of an Ω -3 fatty acid to Ω -6 fatty acid ratio.

HASHIM relates to the prevention of vascular disorders by means of a composition comprising vitamin B6, betaine, choline, vitamin B12 and folic acid. However, this publication also fails to disclose or suggest the claimed long chain polyunsaturated fatty acid fraction.

As the cited publications alone or in combination with each other, fail to even mention or suggest a long chain polyunsaturated fatty acid as set forth in claims 19-34 or 35-38, it is believed that the proposed combination fails to render obvious the claimed invention.

Moreover, applicants traverse the assertion that it would have been obvious to one of ordinary skill in the art, at the time the claimed invention was filed, to combine the claimed components for their known benefit. In addition, applicants traverse the assertion that it would have been obvious to one of ordinary skill in the art to prepare the composition as a nutritional supplement as it was allegedly routine practice to do so in the art at the time the claimed invention was made. It is respectfully submitted that the outstanding Official Action fails to provide any evidence to support these assertions. In fact, applicants believe that it would not have been obvious to one of ordinary skill in the art to combine the teachings of the references or to produce a preparation as set forth in the claimed invention.

In the outstanding Official Action, claims 19-20 and 31 were rejected under 35 USC 103(a) as allegedly being unpatentable over SAGAMI, HORROBIN, HASHIM and SAUVAGE et al. It is believed that the present amendment obviates this rejection.

The SAUVAGE et al. publication is directed to a method for reducing platelet aggregation. It is respectfully submitted

that one of ordinary skill in the art would lack the motivation to combine carefully formulated compound used in a method for reducing platelet aggregation with a nutritional supplement. Moreover, applicants note that SAUVAGE et al. do not teach a long chain polyunsaturated fatty acid as claimed in claims 19-38. As a result, it is believed that SAUVAGE et al. fail to remedy the deficiencies of the above-identified publications.

Claims 19 and 20 were rejected under 35 USC 103(a) as allegedly being unpatentable over SAGAMI, HORROBIN, HASHIM and MURRAY. It is believed that this rejection has been rendered moot by the present amendment.

The MURRAY publication relates to the use of huperzine for treating rheumatic disorders as well as vascular diseases. No mention is made of dementia, cognitive degeneration or hearing loss of their treatment. Moreover, the reference is entirely silent as to the recited fraction of the long chain polyunsaturated fatty acid as set forth in the claimed invention.

Thus, it is believed that the proposed combination of SAGAMI, HORROBIN, HASHIM and MURRAY fails to render obvious the claimed invention.

Claims 19 and 25-29 were rejected under 35 USC 103(a) as allegedly being unpatentable over SAGAMI, HORROBIN, HASHIM, BLAND and CAVAZZA et al. It is believed this rejection has been obviated.

The BLAND publication provides omega fatty acids in

conjunction with vitamins, minerals and other nutrients for reducing coronary heart disease. The CAVAZZA publication relates to a combination of alkanoyl-L-carnitines with omega-3 fatty acids for the prevention and treatment of disorders ranging from cardiovascular disorders to tissue disorders. However, both the BLAND and CAVAZZA publications fail to disclose or suggest dementia, cognitive degeneration or hearing loss. Moreover, the publications fail to even mention the claimed long chain polyunsaturated fatty acids of claims 19-34 and 35-38, respectively. It is believed that the proposed combination fails to render obvious claims 19 and 25-29.

Claims 19 and 30 were rejected under 35 USC 103(a) as allegedly being unpatentable over SAGAMI, HORROBIN, HASHIM, YANAI and HE. The YANAI publication discloses the use of ginkgo tea for preventing and curing vascular diseases. The HE publication teaches that ginkgo components may also be used as a health beverage. However, the YANAI and HE publications fail to disclose the claimed long chain polyunsaturated fatty acids. As a result, it is believed that the proposed combination of SAGAMI, HORROBIN, HASHIM, YANAI and HE fails to render obvious the claimed invention.

In the outstanding Official Action, claims 19, 30 and 32 were rejected under 35 USC 103(a) as allegedly being unpatentable over SAGAMI, HORROBIN, HASHIM, YANAI, BLAND and SAUVAGE. In view of the present amendment, this rejection is

respectfully traversed.

As noted above, the additional publications of YANAI, BLAND and SAUVAGE fail to remedy the deficiencies of SAGAMI, HORROBIN and HASHIM.

In view of the present amendment and the foregoing remarks, therefore, it is believed that this application is now in condition for allowance, with claims 19-38, as presented. Allowance and passage to issue on that basis are accordingly respectfully requested.

Attached hereto is a marked-up version of the changes made to the claims. The attached page is captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE."

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS:

Claim 19 has been amended as follows:

19. (amended) A preparation for the prevention/treatment of dementia syndromes, cognitive degeneration or hearing loss, comprising the following fractions:

fraction a) consisting of long chain polyunsaturated fatty acids containing at least one member selected from the group consisting of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), dihomogammalinolenic acid (DHGLA), arachidonic acid (AA), linoleic acid and α-linoleic acid, wherein the ratio of the total amount of EPA + DHA + DHGLA + AA to the total amount of linoleic acid and α-linoleic acid is above 0.4;

fraction b) consisting of phospholipids, containing at least two different phospholipids selected from the group consisting of phosphatidylserine, phosphati-dylinositol, phosphatidylcholine and phosphatidylethanolamine; and

fraction c) consisting of compounds which are a factor in methionine metabolism, containing at least one member selected from the group consisting of folic acid, vitamin B12, vitamin B6, magnesium and zinc.

Claim 20 has been amended as follows:

20. (amended) A preparation according to claim 19, further comprising a fraction d) consisting of [citrate] citrates

or citric acid.

Claim 22 has been amended as follows:

22. (amended) A preparation according to claim 19, wherein fraction a) consists of [gamma-3] Q-3 and [gamma-6] Q-6 fatty acids.

Claim 23 has been amended as follows:

23. (amended) A preparation according to claim 22, wherein [the gamma-3] Q-3 fatty acids are selected from the group consisting of [eicosapentaenoic acid] EPA and [docosahexaenoic acid] DHA and the [gamma-6] Q-6 fatty acids are selected from the group consisting of [arachidonic acid] AA and [dihomogammalinolenic acid] DHGLA.



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(21) Application Number: 08-165951 (22) Filed: 26 June 1996	(71) Applicant: 000173762 Sagami Chemical Research Center 4-4-1 Nishionuma, Sagamihara, Kanagawa Prefecture, Japan (72) Inventor: K. Yazawa (72) Inventor: K. Watabe (72) Inventor: Y. Otani (72) Inventor: Y. Masuzawa (72) Inventor: E. Tsuji (Personal addresses not transcribed)
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(54) [Title of Invention] BLOOD LIPID IMPROVER AND FOOD ADDITIVE

(57) [Abstract]

[Problem] To provide a DHA derivative effective for preventing and improving vascular diseases such as atherosclerosis.

[Solution] A blood lipid improver and food additive containing a phospholipid incorporating docosa-hexaenoic acid as a constituent fatty acid.

[Advantage] The above blood lipid improver and food additive act to reduce total cholesterol and neutral lipids and raise high density lipoprotein cholesterol more than the DHA-containing triglycerides and ethylesters currently supplied in large quantities.

[Claims]

[Claim 1] A blood lipid improver containing, as its active ingredient, a phospholipid incorporating docosa-hexaenoic acid as a constituent fatty acid.

[Claim 2] A food additive with blood lipid-improving activity, containing a phospholipid incorporating docosa-hexaenoic acid as a constituent fatty acid.

[Detailed Description of the Invention]

[0001]

[Technical field] The present invention relates to a blood lipid improver and food additive containing a phospholipid incorporating docosa-hexaenoic acid as a constituent fatty acid, which acts to improve blood lipids by reducing the levels of factors that cause atherosclerosis such as total cholesterol and neutral lipids, and increasing the level of high density lipoprotein cholesterol.

[0002]

[Prior Art] The n-3 series of higher unsaturated fatty acids, that is, alpha-linolenic acid (ALA), eicosa-pentaenoic acid (EPA), and docosa-hexaenoic acid (DHA), is known to protect against vascular disease such as atherosclerosis. These highly unsaturated fatty acids have been studied in the form of triglycerides or ethyl esters, large quantities of which are readily available. These studies have also indicated differences in their mechanism of action, such as that whereas EPA lowers plasma neutral lipids more than ALA and DHA, DHA lowers plasma cholesterol and phospholipids more than EPA and ALA. Atherosclerosis is a multi-factorial disease and is believed to be promoted by rises in cholesterol and triglycerides and decreases in high density lipoprotein cholesterol as factors in the blood [H. Nakamura, "Lipid Metabolism and Disease / Fat Intake and Morbidity", Saiwai Shobo (1990) pp 385-396]. The development of drugs etc. which would provide still greater improvement in these blood lipids overall is thus to be desired in terms of preventing and treating vascular disease such as atherosclerosis.

[0003]

[Problem to be solved by the invention] The purpose of this invention is to provide a drug with a more effective action and a food additive that improves blood lipids with the aim of improving the blood lipids that cause vascular disease such as atherosclerosis.

[0004]

[Solutions] Upon studying how to solve the above problem, the present inventors discovered that phospholipid containing DHA as a constituent fatty acid – and especially phospholipids containing 10% or more of phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, and/or phosphatidylserine – lower blood total cholesterol and neutral lipids and also act to raise high density lipoprotein

cholesterol more effectively than triglycerides containing the same amount of DHA. They thus completed the present invention.

[0005]

[Embodiments of the Invention] The present invention provides a blood lipid improver and food additive containing a phospholipid incorporating docosa-hexaenoic acid as a constituent fatty acid.

[0006] In terms of effect, it is desirable for the phospholipid of the present invention to be a phospholipid incorporating 10% or more of phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, and/or phosphatidylserine and to contain docosa-hexaenoic acid as a constituent fatty acid.

[0007] What is meant in the present invention by 'improvement in blood lipids' is not only bringing about a fall in the levels of the lipids that cause vascular disease such as atherosclerosis, but also increasing, or at least maintaining, the lipids which protect against such disease, for example, by reducing total cholesterol and/or the level of neutral lipids and also increasing the level of high density lipoprotein cholesterol.

[0008] The phospholipid of the present invention is characterized in that it incorporates DHA as a constituent fatty acid and in terms of activity, it is desirable, in particular, for DHA to account for not less than 5%, preferably not less than 15% and more effectively, not less than 30%. Phospholipid mixtures incorporating DHA as a constituent fatty acid can be obtained by extraction from natural materials such as cuttlefish skin, egg yolk, krill, DHA-producing micro-algae (JP, 7-95875, A) and DHA-producing micro-organisms (JP, 1-199588, A) by conventional methods (JP, 1-131189, 6-321970, 7-68157, A), and may also be obtained by synthesis (for example, JP, 51-91213, 52-89622, 61-129191, A), ester exchange using enzymes [e.g. Y. Totani and S. Hara, J.Am.Oil Chem.Soc., 68, and 848-851 (1991); M. Hosokawa, H. Oshima, H. Kono, Y. Takahashi, M. Hatano, K. Odajima J. Japanese Society of Fisheries Science 59, 309 (1993)] or by integration in bacterial phospholipids [K. Watanabe, C. Ishikawa, H. Inoue, D. Cenhua, and K. Yazawa and K. Kondo, J.Am.Oil Chem.Soc., 71, 325-330 (1994)], etc.

[0009] For example, the main composition of phospholipids extracted from cuttlefish skin is normally 40~70% phosphatidylcholine, 10~30% phosphatidylethanolamine and 5~15% phosphatidylserine. DHA in each constituent fatty acid accounts for phosphatidylcholine 40~60%, phosphatidylethanolamine 15~25% and phosphatidylserine 20~30% and the proportion of DHA in the constituent fatty acids of

the phospholipids as a whole is 25~40%. Phospholipids obtained from cuttlefish skin are therefore a desirable example of phospholipids for the present invention.

[0010] As such DHA-containing phospholipids are amphipathic, they may readily be combined with all foods, whether aqueous or oily and may be widely used added to foods or on their own as health foods.

[0011] The blood lipid improver of the present invention may be administered by oral or parenteral routes to provide the treatment. For oral administration, it can be made into solid preparations such as wettable powders, granules or tablets or liquid preparations such as elixirs. For parenteral administration, it may be made into injections, preparations for mucosal administration or topical preparations. These preparations are manufactured by conventional means by adding physiologically and pharmaceutically permitted excipients/adjuvants to the active ingredient. They may also be made into controlled release formulations by using known techniques. When using said manufacturing adjuvants, the content of the phospholipids in the medication of the present invention is usually 0.1~50% by weight and preferably 0.2~10% by weight.

[0012] The above manufacturing adjuvants used are ingredients appropriate to the route of administration, such as for internal formulations (oral agents), injectable formulations (injections), agents for mucosal administration (buccal agents, troches, suppositories) and topical agents (ointments, medicinal creams, poultices, etc). For example, for oral and mucosal agents, the ingredients used may include excipients (e.g. starch, lactose, crystalline cellulose, calcium lactate, magnesium aluminometasilicate, anhydrous silicic acid, mannitol), binders (e.g. hydroxypropylcellulose, polyvinyl pyrrolidone, etc.), decay agents (e.g. carboxymethyl cellulose, carboxymethyl-cellulose calcium), lubricants (e.g. magnesium stearate, talc), coating agents (e.g. hydroxyethyl cellulose) and taste adjusters; for injections, solvent solutions or adjuvants able to constitute an aqueous injection (e.g. distilled water for injections, physiological saline, propylene glycol), suspension agents (e.g. surfactants such as Polysorbate 80), pH regulators (e.g. organic acids or metal salts thereof) and stabilizers; and for topical formulations, water- or oil based solvent solutions or dissolution adjuvants (e.g. alcohols, fatty acid esters), thickeners (e.g. carboxyvinyl polymers, polysaccharides), emulsifiers (e.g. surfactants) and stabilizers, etc.

[0013] The medication of the present invention with the composition described above may be produced by known manufacturing methods, for example, by the methods

described in the General Rules of the 10th Edition of the Japanese Pharmacopoeia or by appropriately improved methods.

[0014] The dose of the medicinal agent of the present invention will vary depending on the dosage form, but normally, when treating adults, will be 1~1500 mg as DHA. It is desirable for this to be given as 2~3 divided doses over the day. The dose may be adjusted as appropriate to the age, body weight and symptoms of the patient.

[0015] Moreover, the food additive of this invention can be added a variety of foods beginning with soya processed foods such as *tofu* and fermented bean paste; breads, cakes, sponge cakes, biscuits, custard desserts, jellies, ice cream, *konnyaku*; processed fish products such as fishcakes and fish sausage; instant *ramen* and noodles; drinks; dairy products such as cheese, butter or yoghurt; and processed meat products such as hamburgers, hams and sausages. Although the amount added will vary depending on the food, it is normally about 10 mg~10 g as DHA per 100 g.

[0016]

[Examples] The invention is explained in detail below through the examples. However, the invention is not limited to these examples.

[0017] Example 1

Phospholipids extracted from cuttlefish skin were used as the phospholipid. The main composition of these was phosphatidylcholine 63.1%, phosphatidylethanolamine 21.2% and phosphatidylserine 9.1%. DHA in the individual constituent fatty acids accounted for 44.4% of the phosphatidylcholine, 18.0% of the phosphatidylethanolamine and 24.5% of the phosphatidylserine, and the proportion thereof in the constituent fatty acids of the phospholipid as a whole was 33.4%. A triglyceride with the same fatty acid composition as the fatty acid composition of the DHA-containing phospholipid was prepared by combining the triglyceride of each of the fatty acids, for example, DHA triglyceride (DHA 35~50%), EPA triglyceride (EPA 95%), arachidonic (AA) triglyceride (AA 25%) and tripalmitin (palmitic acid 100%). Spontaneously hypertensive rats (SH rats) were divided into four groups of two, and taking a commercial feed to which 1% cholesterol and 0.5% sodium cholate had been added as the base feed, Group A was fed on base feed containing the DHA-containing phospholipid corresponding to 0.7% DHA; Group B on base feed containing the DHA-containing phospholipid corresponding to 0.35% DHA; Group C on base feed containing the DHA-containing triglyceride corresponding to 0.7% DHA; and Group D on base feed containing the DHA-containing triglyceride corresponding to 0.35%

DHA. These feeds were provided as 20 g per day for two weeks. Following the test, blood samples were taken by puncturing the abdominal aorta of the rats whilst under ether anaesthesia, the plasma was separated and the lipids analyzed. The results are shown in Figure 1. Total cholesterol and neutral lipids were lowest in Group A and high density lipoprotein cholesterol was also highest in Group A.

[0018] Example 2

A DHA-containing triglyceride with the same fatty acid composition as the DHA-containing phospholipid was prepared in the same way as in Example 1. SD rats were divided into three groups of six. Group E was fed on the same base feed as in Example 1; Group F on base feed containing the DHA-containing phospholipid corresponding to 0.7% DHA; and Group G on base feed containing the DHA-containing triglyceride corresponding to 0.7% DHA. These feeds were given as 24 g per day for two weeks. Following the test, blood samples were taken by puncturing the abdominal aorta of the rats whilst under ether anaesthesia, the plasma was separated and the lipids analyzed. The results are shown in Figure 2. Total cholesterol and neutral lipids were lowest in Group F and clearly lower than in the control group E. Conversely, high density lipoprotein cholesterol was highest in Group F and higher than in Group E. The fall in total cholesterol and rise in high density lipoprotein cholesterol seen in Group F were also statistically significantly greater than in Group E and in Group G, which had received a triglyceride containing the same amount of DHA.

[0019]

[Effect of the Invention] The blood lipid improver and food additive of this invention act to reduce total cholesterol and neutral lipid levels and raise the high density lipoprotein cholesterol level, and so can be used to prevent or improve vascular disease such as atherosclerosis.

[Brief explanation of drawings]

Figure 1 Graph illustrating mean and standard deviation for each group in blood total lipids (TC), neutral lipids (TG) and high density lipoprotein cholesterol (HDL-C) analyzed in Example 1.

Figure 2 Graph illustrating mean and standard deviation for each group in blood total lipids (TC), neutral lipids (TG) and high density lipoprotein cholesterol (HDL-C) analyzed in Example 2.

Figure 1

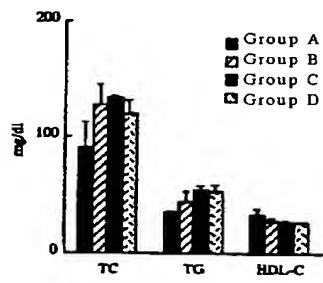


Figure 2

